Specific support for the new claims can be found in the specification as follows:

Claims 31 and 39:	Page 8, lines 2-27; page 10, line23 to page 11, line 7.
Claims 32 and 40:	Page 8, line 28 to page 9, line 8.
Claims 33 and 41:	Page 9, lines 9-23; page 11line 8 to page 12, line 12.
Claims 34 and 42:	Page 9, line 24 to page 10, line 10.
Claims 35 and 43:	Page 10, lines 11-22.
Claims 36 and 44:	Page 28, line 28 to page 34 line 8.
Claims 37 and 45:	Page 34, line 9 to page 38, line 8.
Claims 38 and 46:	Shows the case the specific lectin and antibody are contained mixed at a time.
Claim 47:	Page 6, line 28 to page 7, line 2.
Claim 48:	Page 6, lines 7-24.

The following chart summarizes the main features of the independent claims:

Claim Summary

Independent Claim	Determining Two Types of Tg	Measuring Malignancy of Thyroid Tumor	Reagent	Non-competitive Binding	Competitive Binding
19	√				
21	√				√
22		√			
27			√		
28			√		
30		√			
31	√			√	
32	√			NA	NA
33	√				√
34	√				√
35	√				√
36	√			NA	NA
37	√			√	
38	√				√
39		√		√	
40		√		NA	NA
41		√			√
42		√			√
43		√			√
44		√		NA	NA
45		√		√	
46		√			√

To assist the Examiner in examining these claims, the applicants would like to point out the following:

1. Claims 31-38 correspond to claims 39-46 with the difference being that claims 39-46 are directed toward the overall method for determining malignancy of a thyroid tumor.

2. Claims 31, 37, 39 and 45 recited a non-competitive binding scheme. A non-competitive binder is, for example, an anti-thyroglobulin antibody-1 capable of binding to all thyroglobulin.

3. Claims 21, 33-35, 38, 41-43 and 46 recite a competitive binding scheme. A competitive binder is, for example, an anti-thyroglobulin antibody-2 capable of binding to Tg but not capable of binding to Tg to which the specific lectin or the specific antibody is already bound.

4. To further assist the Examiner attached Appendix A includes flowcharts of the method claimed in claims 31-46. Please note that as described in the nine working modes of the present invention on pages 8-12 of the specification the particular order of adding materials to the solution is not important.

5. To further assist the Examiner in analyzing the claimed invention with respect to the disclosure of the cited references, Appendix B includes flowcharts of the methods disclosed in the cited references. While the applicants understand that Appendix A and Appendix B contain a lot of information, the flowcharts should provide an easier way for analyzing the disclosed methods.

In response to the Office Action, the applicants make the following points with respect to the following numbered paragraphs in the Office Action.

2-4. The claims have been amended to address each of the 35 U.S.C. §112 rejections noted by the Examiner.

5-9. Each of these specific rejections under35 U.S.C. §102(b) is addressed below.

10-11. The claims have been amended to address these two specific rejections.

12-13. The claims have been amended and new claims added to positively recite a complete method.

14-17. Claim dependency has been changed and the claims have been amended to address all of the points raised by the Examiner.

18-19. The rejection of the claims under 35 U.S.C. §103 is addressed below.

Maruyama et al.

Claims 4, 10-11 and 15-16 stand rejected under 35 U.S.C. §102(a) as being anticipated by this reference.

The verified English translation of the priority document is being submitted herewith. The application now predates the reference, thus removing it.

SAR van de Graaf et al.

Claims 19, 21 and 27 are rejected under 35 U.S.C. §102(b) as being anticipated by this reference.

In the reference, an in vitro eukaryote expression system for human Tg cDNA is developed. Four overlapping Tg cDNA constructs were prepared using human thyroid DNA as a template and were transiently transected in a human thyroid cell (eukaryote). And the lysate of the transected cell is subjected to the examination (p. 509, col. 1, lines 1-11).

The Office Action states:

> (1) The reference clearly states that Tg proteins were immunoprecipated and total Tg isolated using RaHuTg, which is an anti-Tg antibody.

However, 'immunoprecipitated' does not mean 'the measurement of Tg.' In the reference, four overlapping Tg cDNA constructs were prepared and were transected in a human thyroid cell lines. Expressed Tg fragment protein obtained from the transected cells and the Tg fragments were *immunoprecipitated* using *RaHuTg to select the expressed proteins having the antigenicity as Tg, but the amount of Tg was not measured* (p. 510, col. 2, lines 16-23, *Immunoprecipitation*).

The Office Action states:

> (2) These isolated Tg were the analyzed and detected with 4 different lectins.

However, the "analyze" and "detect" means the *analysis and detection of glycosylation* of Tg (*i.e.* sugar chain structures of Tg), but does not means the measurement of Tg amount. After immunoprecipitation, immunoprecipitated expressed Tg protein fragment is subjected to the glycosylation differentiation using lectins and antidigoxigenin conjugated to alkaline phosphatase (p. 510, col. 2, lines 25-37). Tg of the reference is not Tg protein itself, but is a fragment of Tg (Fig. 1, Table 1, Fig. 2, p. 512, col. 1, lines 3-10).

In the reference, expressed Tg in eukaryotic cells is obtained, and its antigenicity is determined by the cross-reaction test using anti Tg antibody (immunoblotting), and glycosylation differentiation is determined on the basis of the property of binding to lectins. Thus, anti-Tg antibody and lectin are used to confirm that the obtained recombinant Tg has the same property as the human Tg (p. 512, col. 1, lines 3-27). Immunoblotting using RaHuTg is performed in order to identify th fraction separated by SDS-PAGE, but not to measure Tg amount (p. 510, col. 2, *Immunoblot analysis*). The use of both antibody and lectin to measure the amount of Tg is not disclosed in the reference.

Hanham et al.

Claims 4, 10-11, 15-16, 19-25 and 27-28 stand rejected under 35 U.S.C. §102(a) as being anticipated by this reference.

The object of the reference is to determine the sugar chain structure of Tg. In the reference, Tg is modified on its carbohydrate structure by enzymes, the modified Tg is subject to

pectin affinity electrophoresis using lectin gel or electrophoresis using antibody gel, and the gel is subjected to dying using Coomassie blue.

The Office Action states:

> (1) The reference clearly states that an anti-Tg antibody was used as part for the measurement (p. 163, col. 2) and
> (2) These isolated Tg were "quantified" (see, for example, abstract and figures 1-6).

However, as is clear from Figs. 1-6 and p. 160, col. 2 and "Results," lines 1-4, the quantification of Tg is not actually shown. In the reference, known amount of Tg is applied to the gel (Fig. 4, Fig. 5), but unknown amount of Tg is not applied to the gel. Therefore, the reference, the amount of Tg in the sample is not measured. Furthermore, on page 163, the result of electrophoresis using a lectin gel *or* antibody gel is shown (Fig. 5). However, the measurement using both lectin and antibody is not shown.

The Office Action states:

> (3) These determinations are useful for determining thyroid disease, including thyroid malignancy (page 158, abstract and column 2).

However, the "determination" means "determination of glycosylation." On lines 2-4 of the Abstract, the reference says, "modifications of Tg glycosylation occur in various thyroid disorders. In order to study possible changes in *glycosylation of tissue Tg associated with thyroid disease,* ...." Further, the reference says, "There is some evidence that Tg glycosylation is modified in various thyroid disorders. Characteristic changes ..." (P. 158, col. 2, lines 3-5). That is, the reference discloses that *determination of glycosylation* is useful for determination of

thyroid disease, but not discloses that the *amount of Tg* is useful for determination of thyroid disease.

Additionally, Tg amount in the sample from body fluid is not disclosed in the reference. And, the use of both antibody and lectin to measure the amount of Tg is not disclosed.

Tarutani et al

Claims 4, 10-11, 15-16, 19-25 and 27-28 stand rejected under 35 U.S.C. §102(b) as anticipated by this reference.

Basically, soluble protein extracted in the buffer is obtained, and the protein is regarded as Tg in the reference. Therefore, the determined protein is not equal to Tg itself (for example, p. 852, col. 1, *Thyroid Glands* and *Isolation of Thyroglobulin on a Concanavalin A-Sepharose Column*).

The Office Action States:

> (1) the reference clearly states that an anti Tg antibody was used as part for the measurement (p. 854, col. 1). (2) these isolated Tg were "quantified."

However, page 854, an anti-human Tg rabbit serum is used only for testing cross-reaction by examining the precipitin line with an anti-human Tg rabbit serum (p. 854, col. 1, lines 26-31). The procedure is not a part for the measurement. The procedure is not related to the measurement.

The Office Action states:

> These isolated Tg were "quantified" (see, for example, Tables I and II).

However, Tg concentration in Tables I and II are measured allowing the procedure of the reference. However, as mentioned above, in this reference, soluble protein extracted in the buffer from the thyroid tissue is regarded as Tg and the amount of the protein is measured by absorbance of 280 nm (p. 852, col. 1). That is, in the reference, Tg itself is not measured, and Tg is calculated as protein simply, and the method is not quantitative measurement.

The Office Action states:

> (3) These determinations are useful for determining thyroid disease, including thyroid malignancy (see for example, Table I and II).

However, "determination" is "determination of affinity to pectin is useful for determining thyroid disease" (abstract, lines 11-20, p. 854, col. 1, lines 1-13). But there is found no disclosure in the reference concerning that the measurement of Tg amount is useful for determining thyroid disease.

Wang et al.

Claims 10-11, 15-16, 19-22, 24-25 and 27-28 stand rejected under 35 U.S.C. §102(b) as anticipated by this reference.

In the reference, pectin-binding rate of WGA etc. to carcinoma cell etc. is determined. It means that the sugar chain structure of cell membrane is examined.

The Office Action states:

> (1) The reference teaches immunohistochemical detection, and use of an anti-Tg antibody for detection of Tg is an art standard from of immunohistochemical detection as set froth in the references above. (2) These isolated Tg were "quantified." (3) These determinations are useful for determining thyroid disease, including thyroid malignancy.

However, the "determination" in this reference means that the determination of *immunoreactivity of Tg of thyroid tissue or cells* and determination of *lectin binding activity on the surface of thyroid tissue or cells.*

On lines 8-10 of the Abstract, the reference says, "the positive rate of Tg immunoreactivity was significantly different between these three kinds of tumor" and "Some Gastrin, SS and calciotonin positive cells were also recognized in *carcinoma of thyroid* on lines 11-13." That is "immunoreactivity" to antibody of Tg of the different thyroid tissue or cell is examined.

The Abstract says, "Lectin-binding rate of normal thyroid tissue revealed that thyroid carcinoma and 9 cases of normal thyroid tissue revealed that different lectin had a selective binding activity to various types of thyroid carcinoma and normal thyroid cells" (Abstract, lines 13-16). That is, lectin binding rate to thyroid tissue or cell is determined. Lectin is binding to the surface of thyroid tissue or cell. And, the reference says also, "From the date obtained, it seemed that the *morphological differentiation* of thyroid carcinoma was in correspondence with difference of function ..." (Abstract, lines 17-18). Measurement of Tg amount using pectin is not disclosed in the reference.

After all, the immunoreactivity of Tg of thyroid tissue or cell and pectin binding rate of thyroid tissue or cell are examined, but the measurement of Tg amount in the sample from body fluid is not disclosed in the reference. And the use of both antibody and pectin to measure the amount of Tg is not disclosed.

Claims 4, 10-11, 15-16 and 19-29 stand rejected under 35 U.S.C. §103(a) as being unpatentable over **Maruyama et al., SAR van de Graff et al., Hanham et al., Tarutani et al.,** or **Wang et al.** in view of **Larena a et al., Langenbecks Archiv fur Chrugie** Vol 381/2 pages 102-113 1996.

In any of the references, there is no disclosure concerning measurement of *the amount* of Tg, and there is no disclosure concerning *the ratio* of the amount of Tg having a specific sugar chain/the mount of total Tg. Therefore, all of the claims, of the present invention are novel and unobvious over the cited references.

Simply by comparing the flowcharts of the methods disclosed in the cited references (Appendix B) with the flowcharts of the applicants' claimed method, no conclusion of obviousness can be drawn because of the specific lack of teaching of the applicants' method steps. Without more, a mix of references each measuring different quantities cannot be combined to suggest determining the malignancy of a thyroid tumor. Simply put, nowhere in the references is there disclosed a method based on the measurement of Tg which is a method for determining an amount of one of two types of Tg or a method for measuring a malignancy for thyroid tumor.

Based on the above showing, the applicants' claimed method is not anticipated or even suggested by the references it is respectfully requested that all claims be passed to issue.

If, for any reason, it is believed that this application is not now in condition for allowance, the Examiner is requested to contact Applicant's undersigned attorney at the telephone number indicated below to arrange for an interview to expedite the disposition of this case.

In the event that this paper is not timely filed, Applicant respectfully petitions for an appropriate extension of time. The fees for such an extension or any other fees which may be due with respect to this paper, may be charged to Deposit Account No. 01-2340.

Respectfully submitted,
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McLELAND & NAUGHTON

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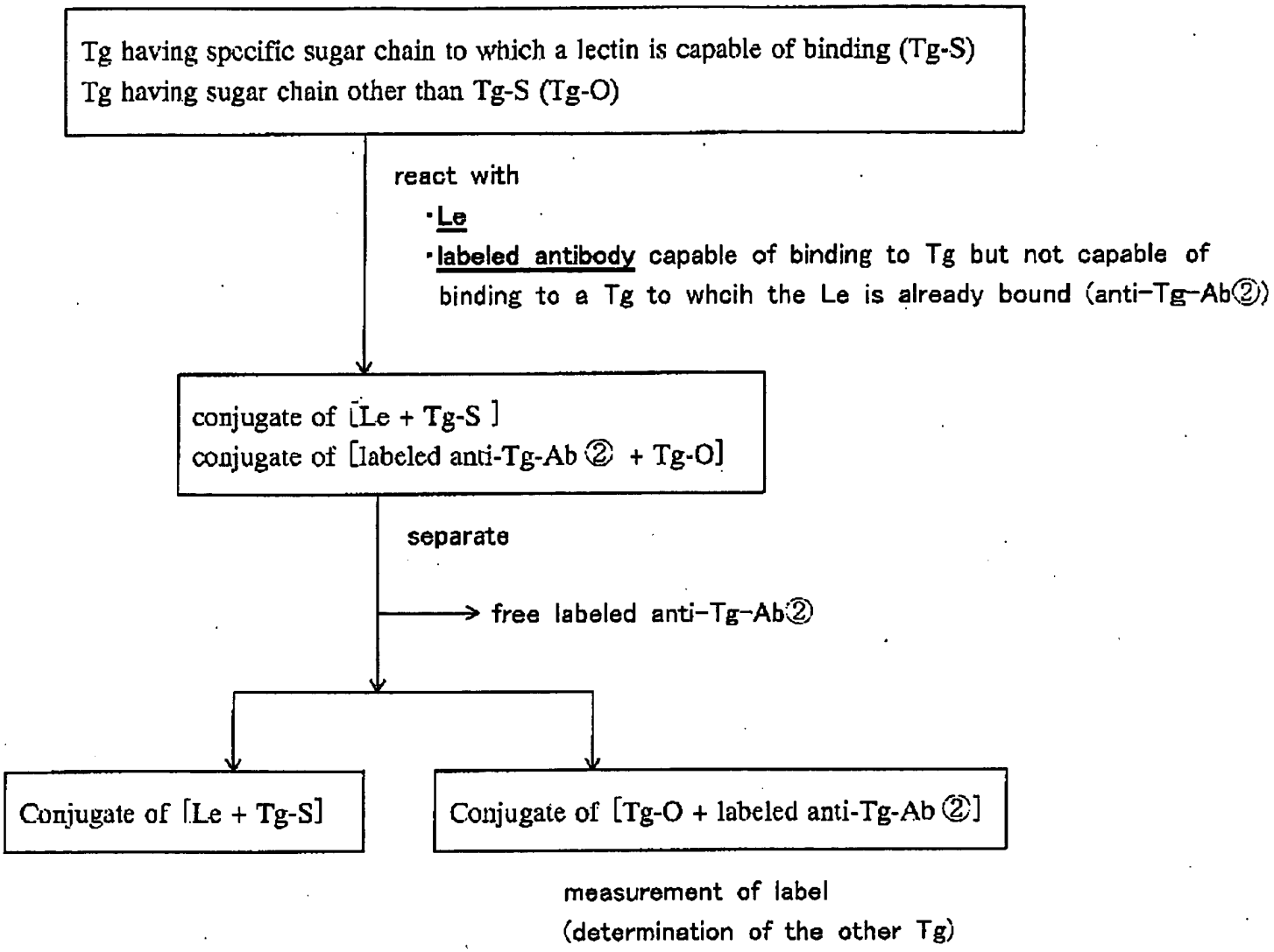
Enclosures: Appendix A and B

Claims 35, [redacted] 43

(p.10, lines 11-22)

sample

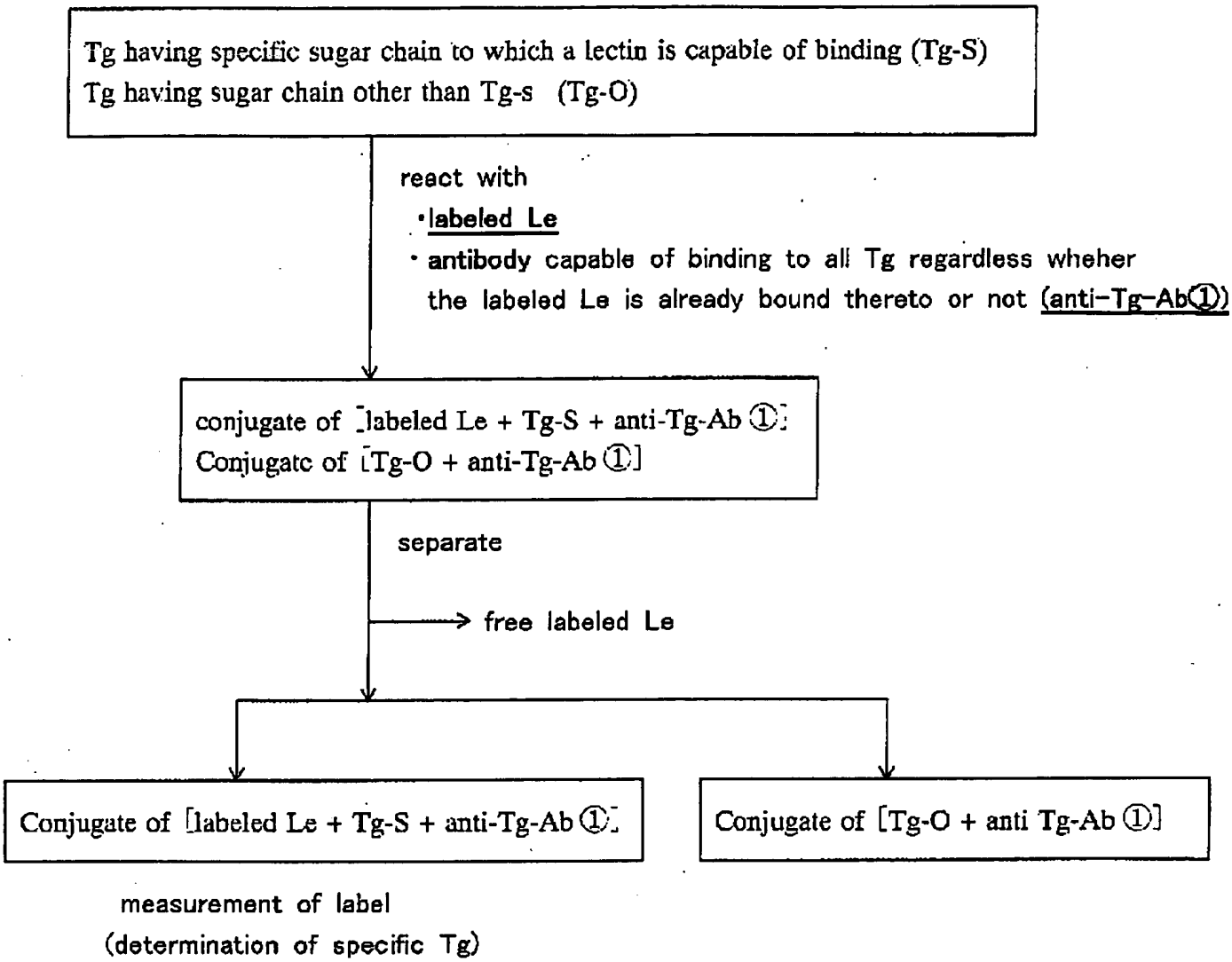


Appendix A 1/5

Claims 31, 39

(p. 8, lines 17-27)

sample

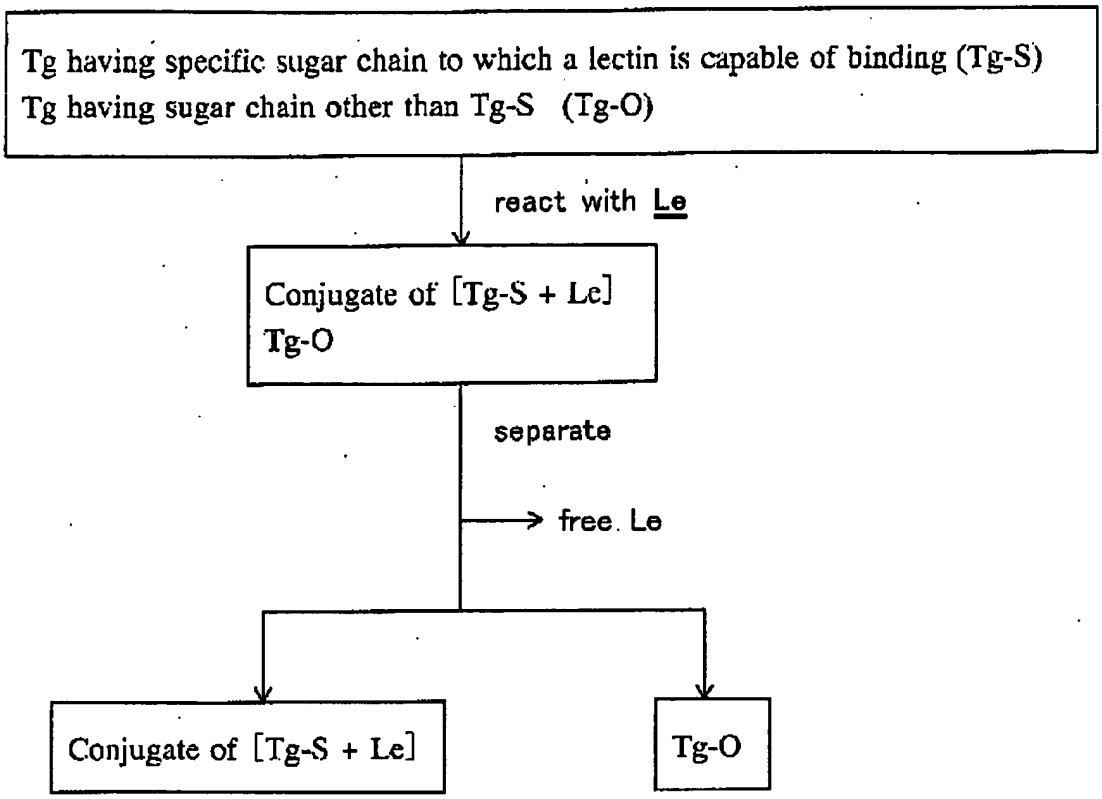


Appendix A 2/5

Claims 32, 40

(p. 8, line 28-p. 9,line 8)

sample

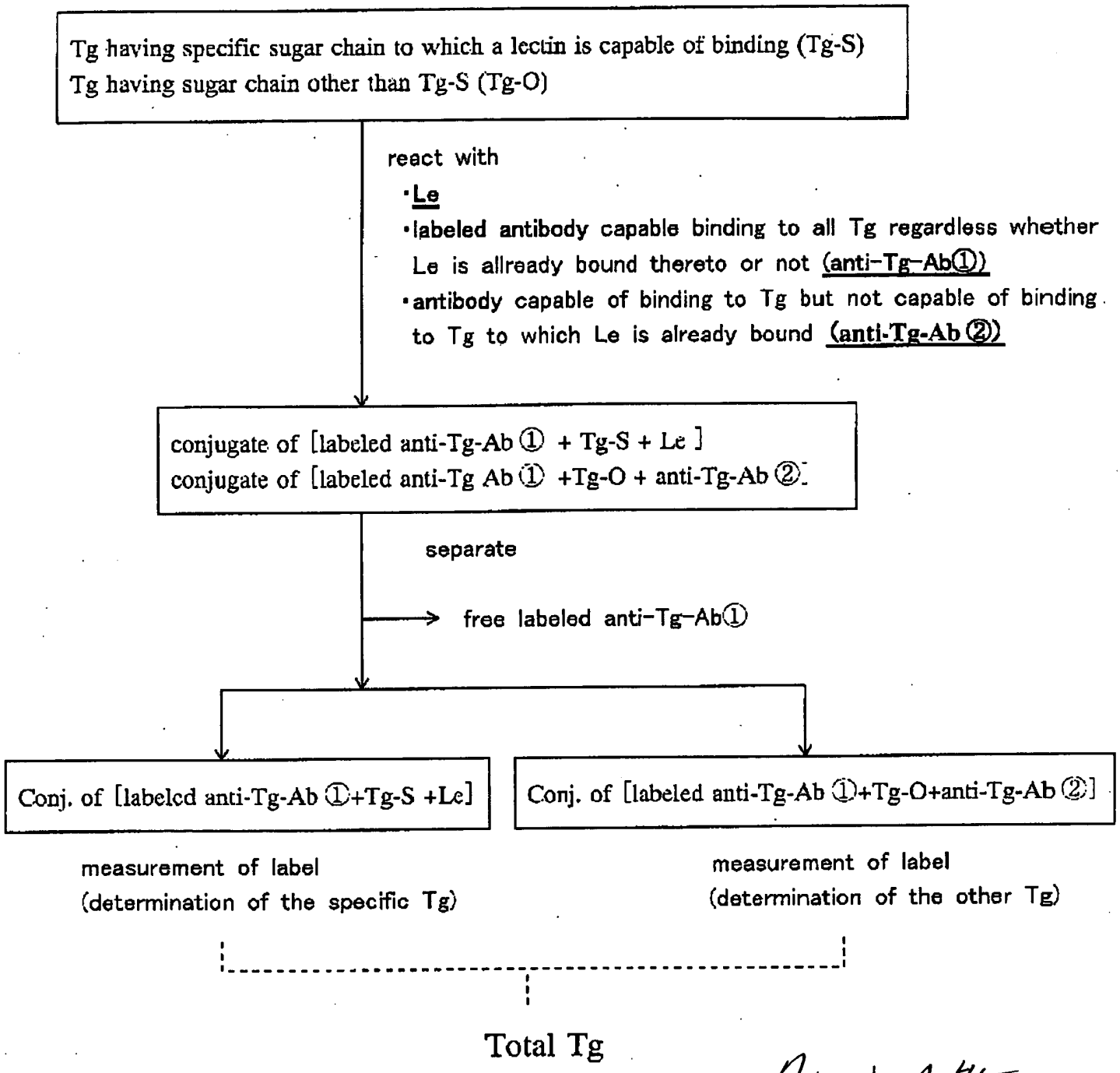


Appendix A 3/5

(p.11, lines 8-24)

Claims 33, 41

sample

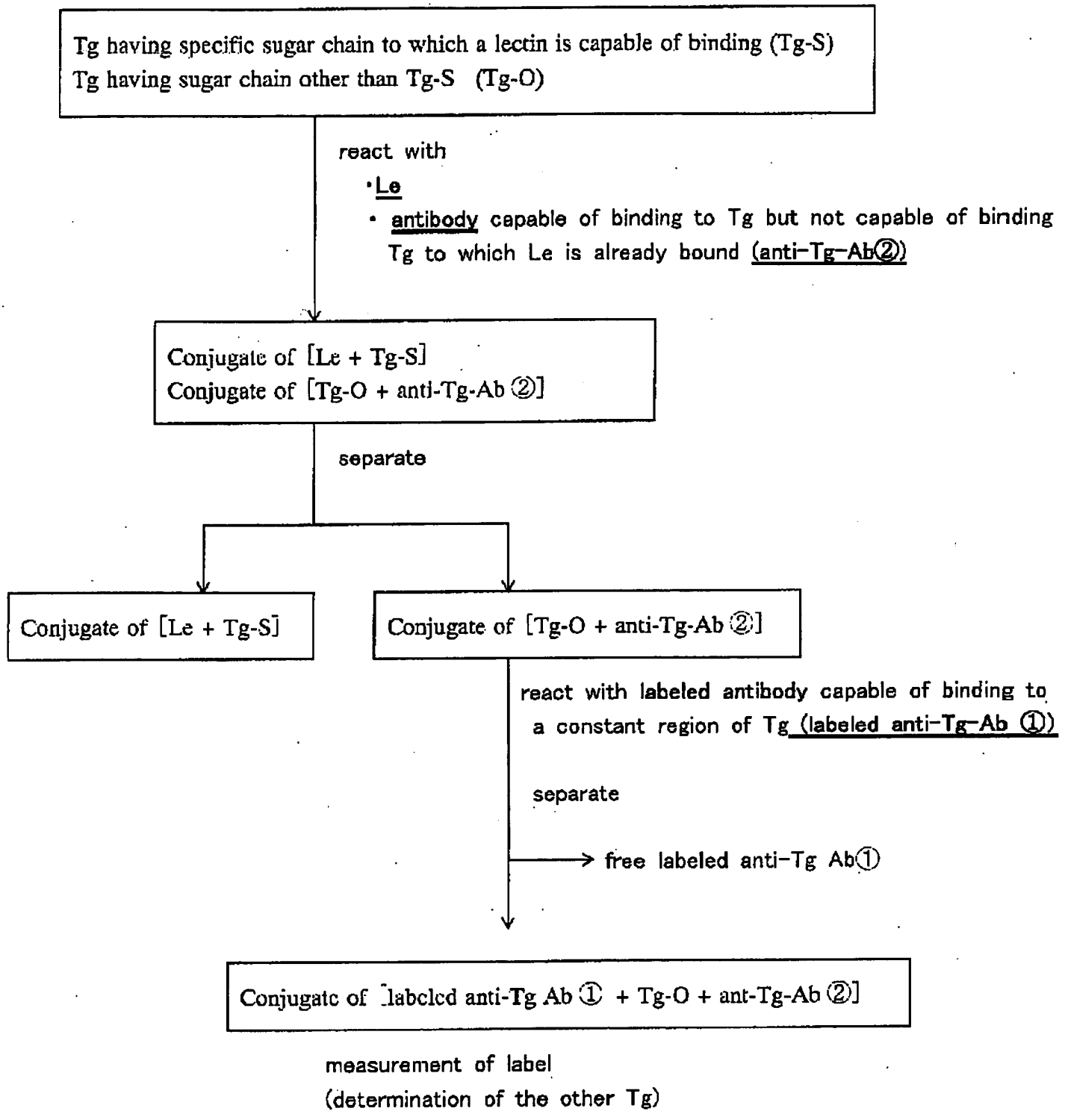


Appendix A 4/5

(p. 9, line 24-p.10, line 10)

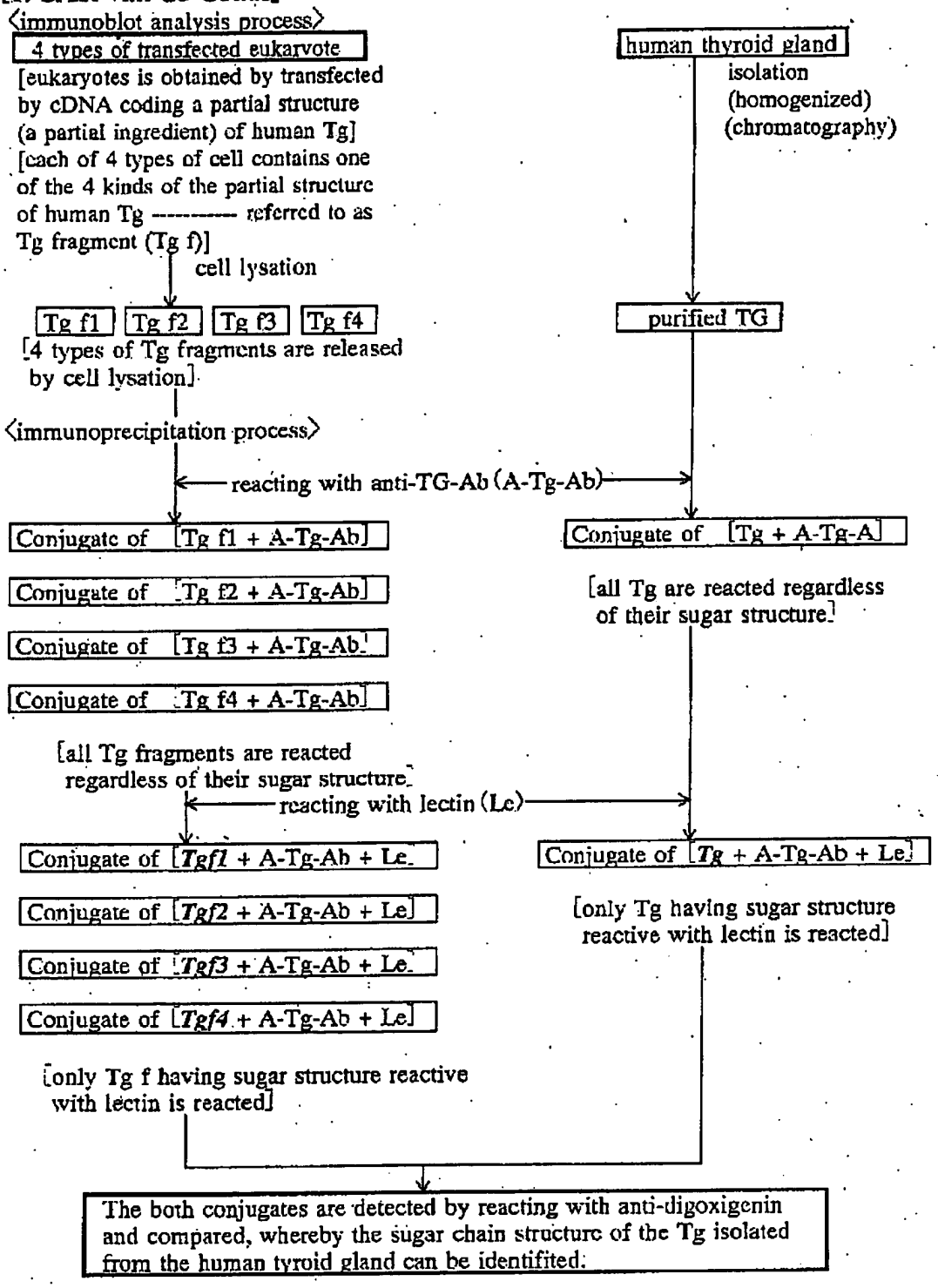
Claims 34, 42

sample



Appendix A 5/5

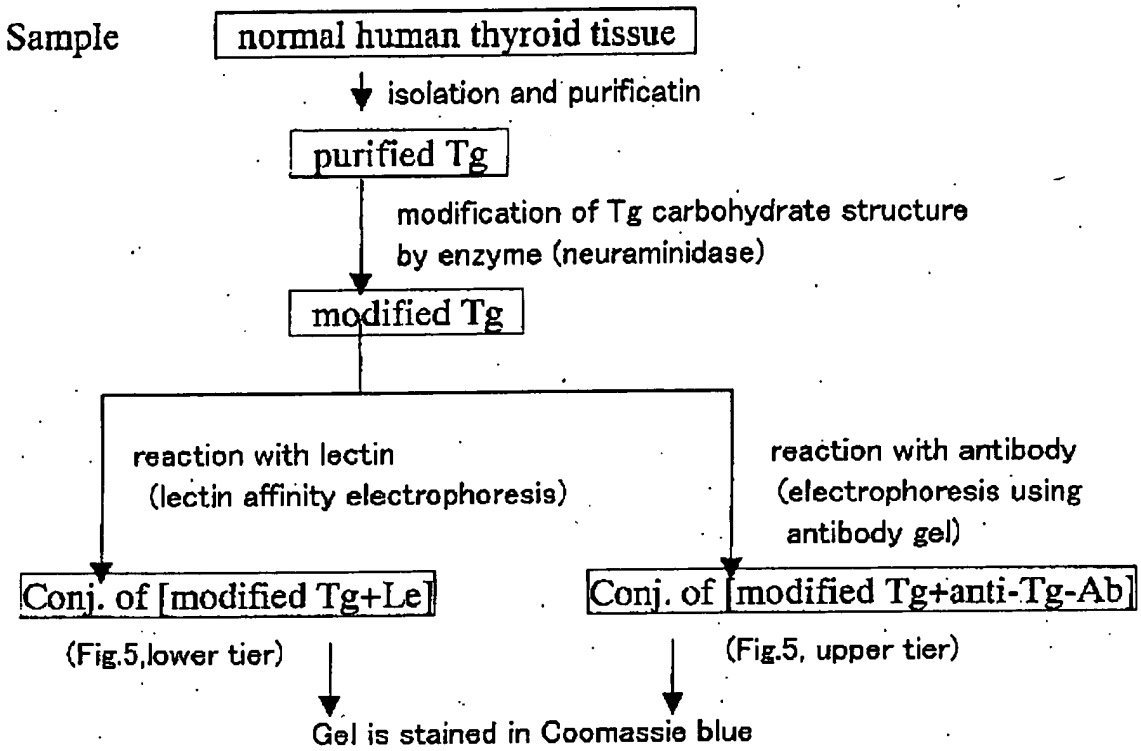
[1. SAR van de Graaf]



Conjugates of [Tg+anti-Tg antibody] and [Tg+anti-Tg antibody+lectin] are obtained (conjugate of [Tg+lectin] is not obtained), however, those reactions are "qualitative", never "quntitative"; at least actual amounts of those conjugatesare not measured (needless to say, actual amount of Tg contained in the sample, human tyroid gland, is not measured). This is because of fundamental difference in the inventive concept or the purpose of technology between the reference and the present invention (the former being identification of sugar chain structure in Tg, the latter being amounts f the total Tg and the specific Tg in a living sample).

Appendix 13 1/4

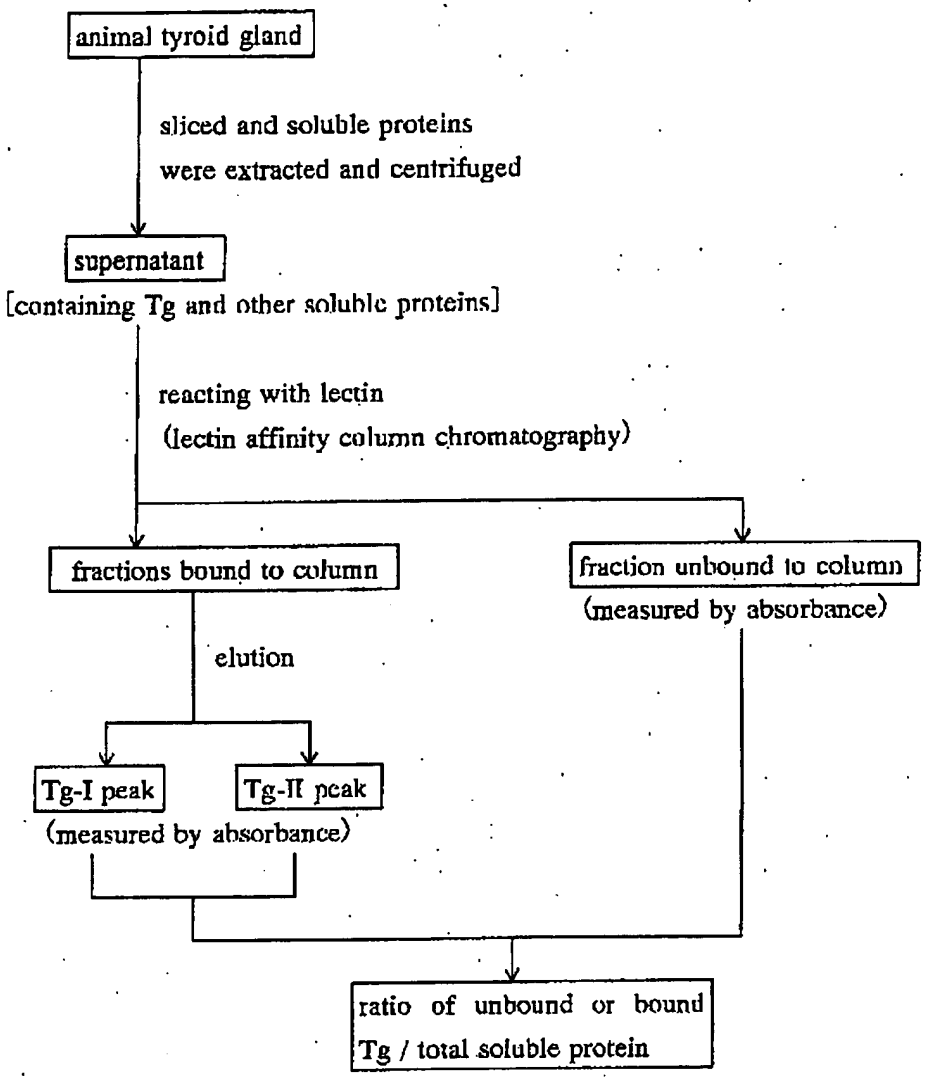
[2. Hanham et al]



The sugar chain structure of the Tg is examined by the binding activity to the lectin.
The antigenecity is determined by the binding to the antibody.
Conjugates of [modified Tg+Le] and Conjugate of [modified Tg+anti Tg-Ab] are obtained but conjugate of [Tg+anti Tg-Ab+Le] is not obtained.
The quantification of Tg is not shown.

Appendix 13 2/4

[3. Tarutani et al]



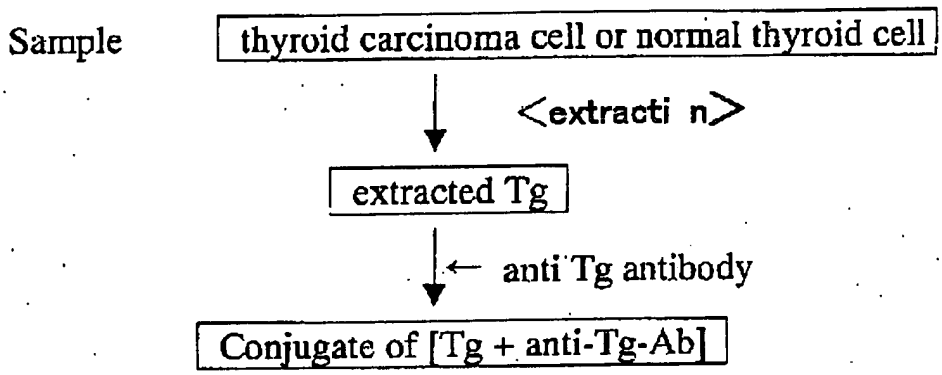
As to the anti-human Tg rabbit serum, there is found only such disclosure as "this material (i.e. unbound fraction) yielded a precipitin line with ------an anti-human Tg rabbit serum". Namely, the disclosure concerned is nothing but meaing that unbound fraction is reactive with anti-human Tg rabbit serum, and no other disclosure is found concerning for instance the next process or treatment to be applied to the unbound fraction or reaction product of the serum with the fraction.

In any event, no disclosure is found concerning measurement of an amount of reaction product of Tg with anti-Tg antibody (an amount of Tg reactive with anti-Tg antibody).

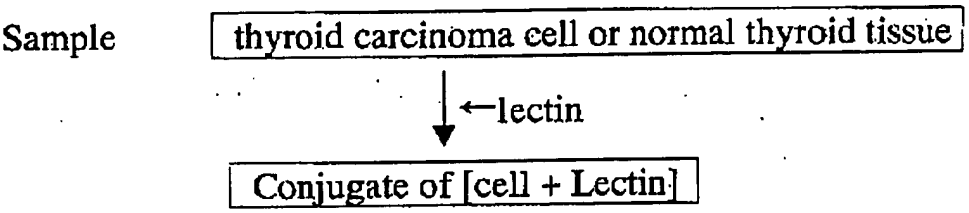
Appendix B 3/4

[4. Wang et al.]

1. Examination of **immunoreactivity**



2. Examination of **lectin binding**



[5. Larena A et al.]